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Human insulin from a precursor overexpressed in the methylotrophic yeast *Pichia pastoris* and a simple procedure for purifying the expression product.

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Wang Y, Liang ZH, Zhang YS, Yao SY, Xu YG, Tang YH, Zhu SQ, Cui DF, Feng YM.

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The methylotrophic yeast *Pichia pastoris*, which proved successful in producing many heterologous proteins, was used to express an insulin precursor. A transformant with a high copy number of the gene integrated into the chromosome was obtained by the dot-blotting method. In high-density fermentation using a simple culture medium composed mainly of salt and methanol, the expression level reached 1.5 g/L. A simple two-step method was established to purify the expression product from the culture medium with an overall recovery of about 80%. After tryptic transpeptidation, human insulin with full receptor binding capacity and biological activity was obtained. In the presence of zinc, the recombinant human insulin could be crystallized in the rhombohedral form. Copyright 2001 John Wiley & Sons, Inc.

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